Routine Screening for Chlamydia Trachomatis in Subfertile Women – Is it Time to Start?

C T Yeong, T L W Lim, R Lin, S Y Se Thoe, N Leong

ABSTRACT

Genital *Chlamydia trachomatis* infection has long been recognised as the major cause of pelvic disease and subsequently infertility. The diagnosis of this infection has traditionally relied on tissue culture. The availability of DNA amplification methods like ligase chain reaction promises faster and more sensitive results. This study was conducted to evaluate the prevalence of *chlamydial infection* in a subfertile population subgroup.

Aim: A case control longitudinal study of 100 subfertile women in a tertiary teaching hospital were analyzed for the prevalence of genital *Chlamydia trachomatis* infection using ligase chain reaction test kit.

Results: A prevalence rate of 8% was detected, the majority being 25 years old or less (33.3%), p = 0.007. All patients gave no prior history of abnormal PAP smears, hospitalisation for pelvic inflammatory disease or abnormal vaginal discharge at the time of investigation.

Conclusion: Our infertile group of patients has a relatively high incidence of silent genital *Chlamydia trachomatis* infection. This being highest in the below 25 years old age group. This finding indicates that screening for chlamydia may be necessary for the subfertile couple presenting to clinic. This is especially so if the patient is of the younger age group.

Keywords: chlamydia, infection, subfertile, prevalence, pelvic

Department of Reproductive Medicine
KK Women's and Children's Hospital
100 Bukit Timah Road
Singapore 229899

C T Yeong, MRCOG (Lond), MMed (O&G), MRACOG (Aust)
Registrar

T L W Lim, MBBS
Medical Officer

R Lin, FRCPA (Aust), MSc (Lond), FAMS
Consultant Microbiologist

N Leong, GRCOG (Lond), MMed (O&G), FAMS
Head and Senior Consultant

Department of Pathology
Singapore General Hospital
Outram Road
Singapore 169608

S Y Se Thoe, MSc
Scientific Officer

Correspondence to:
Dr C T Yeong

INTRODUCTION

With an estimated 90 million cases worldwide[1] including an estimated 4 million new infections occurring each year in the United States, *Chlamydial* infections are the most prevalent of the sexually transmitted bacterial infections[2]. Untreated genital *Chlamydial* infections can lead to serious complications including pelvic inflammatory disease, infertility, ectopic pregnancies and neonatal infections.

Nucleic Acid amplification technology in the form of ligase chain reaction (LCR) has proven itself to be the new gold standard in detecting *C. trachomatis*[3-5]. The sensitivity of testing by LCR is markedly greater than that by culture. A number of studies have shown a sensitivity for amplification assays about 20% to 40% higher than cell culture or antigen detection methods (50% – 80 %)[4,5]. The sensitivity of the ligase chain reaction (LCR) has been reported to be 96.1% with the specificity to be 99.6% . Here, we performed a pilot study to evaluate the prevalence of *C. trachomatis* using LCR in 100 consecutive subfertile Singaporean women.

In our pilot study, cervical specimens chosen for analysis over first void urine specimens because all the women would required a pelvic examination as part of their consult and furthermore as the organism is intracellular, this could mean better sensitivity.

MATERIAL AND METHOD

One hundred consecutive women attending the subfertility clinic in KK Women's and Children's Hospital were enlisted in the study.

Endocervical swab specimens were taken with the LCx STD Swab Specimen collection and Transport Kit from Abbott Laboratories and analysed. Collection of endocervical samples involves the insertion of a cotton or dacron swab or metal shaft (or a brush) at least 2 mm into the opening. The instrument is rotated to strip off epithelial cells which are infected with chlamydia. It is important that an endocervical specimen is obtained rather than a high vaginal specimen because this bacteria infect the columnar epithelial cells of the cervix, but do not infect the keratinised, squamous epithelial cells of the ectocervix or vagina. The specimen is then transported in the commercially prepared kit to the laboratory. The LCR method essentially exploit the natural DNA modifying activities of various enzymes to allow in-vitro amplification of a specific nucleic acid sequence of interest which are then detected via photometric colour detection.

Statistical significance performed using Fisher's exact test and the independent *t* test.

RESULTS

1. Eight patients were detected to have asymptomatic genital *Chlamydia trachomatis* infection.
   The mean age of all patients was 31.9 years old (range 19 to 42).

The mean age of husbands in our study was 35 (range 25 to 45).

10 patients have had a previous marriage.

2. The patients that were tested positive were at a significantly younger age compared to those that were tested negative (Table I).
3. The patients that are below 25 years old have a significantly higher chance of contacting this infection (Table II).
4. Amongst the 100 consecutive women, 66 patients presented with primary subfertility, but this factor is not shown to have any correlation with the risk of infection (Table III).
5. There was no recorded history of pelvic inflammatory disease, abnormal mucopurulent vaginal discharge or abnormal Pap smear.

Table I – Mean age of patients

	Frequency	%	Mean age (in years)
Test positive	8	8	25.1
Test negative	92	92	32.4

(*t*-test, $p < 0.001$)

Table II – Age of patients tested positive

Age	Tested positive	Total No.	%
≤ 25	4	12	4/12 (33.3%)
> 25	4	88	4/88 (4.5%)

Fisher's exact test, $p = 0.007$)

Table III – Patients with primary and secondary subfertility

	Primary subfertility	Secondary subfertility	Total
Test positive	6	2*	8
Test negative	60	32	92

(Fisher's exact test, $p > 0.05$)

* 2 patients with history of induced abortion

DISCUSSION

The role of *Chlamydia* in infertility is well documented[6,7]. In fact, *Chlamydia trachomatis* is the organism most frequently associated with infective tubal damage. Infertility occurs in as many as 20% of women who have had a single episode of salpingitis and the risk of sterility increases to 40% in women who have had three or more episodes. Retrospective serologic surveys of women with ectopic pregnancies and tubal factor infertility have found many, if not most, of these women to have evidence of prior *Chlamydia* infection. Recently, Keay and co-workers[8] from Bristol reported that poor response to gonadotropin stimulation was associated with a higher prevalence of IgG antibodies to *Chlamydia trachomatis* and *C. trachomatis* infection and its sequelae may directly affect ovarian function.

As the number of reported cases of genital *Chlamydia trachomatis* increases in countries like the USA and the UK[7], it is time that the epidemiology and scope of this problem in our society be quickly addressed too.

Traditionally, in vitro diagnostic methods for infectious disease include culture, complement fixation assay, radioimmunoassay (RIA), enzyme immunoassay (EIA) and DNA probe technology. Recent studies have shown that the sensitivity of testing cervical swabs by LCR was markedly greater than that by culture[5,8] and EIA. This increase in sensitivity is based on the principle of nucleic acid amplification where cycles of denaturation, probe hybridisation and enzymatic manipulation are utilised to replicate the nucleic acid sequence of interest.

In addition to its chromosomal DNA, *C. trachomatis* harbours a cryptic plasmid, which is found in all serovars at approximately 10 copies per reticulate body. The LCR target is located within this plasmid and in a short sequence which is highly conserved among all the serovars of *C. trachomatis* but is not found in other species. We have found the LCR to be simple, non-invasive, quick (results are available on the same day) and requiring minimum operator intervention. A further advantage that this LCR kit offers is that the medium for transport can be stored at the clinic in room temperature. This is in contrast to the traditional culture method which requires the medium to be kept in the laboratory and strict storage and transport instructions are needed to ensure viability of the organism. Tissue culture also suffers from the problem of inter-laboratory variability ie. the sensitivity of the culture diagnosis is dependent on how well trained the microscopists are, as well as how sensitive the culture system that the laboratory uses. As such, there are only a few laboratories that have both the facilities and expertise to perform tissue culture diagnosis for *C. trachomatis*.

Our small study revealed a prevalence rate of 8% amongst those that visited the subfertility clinic. A similar study performed in the Department of Reproductive Medicine between October 1993 to March 1994, when 100 consecutive subfertile women were screened using the Bio sign™ *Chlamydia* test, the prevalence was 5% (unpublished data). The BioSign™ *Chlamydia* test involves the chemical extraction of the *chlamydia* antigen (liposaccaride, LPS) followed by solid phase immunometric assay technology for the qualitative detection of extracted LPS. Although this could be due to the more sensitive diagnostic tests, the possibility of an actual increase as a result of the changes in sexual morals cannot be ignored. Another reason why newer methods like the ligase chain reaction (LCR) have better sensitivities as compared to the conventional culture methods could be due to the inhibition of *Chlamydia* by host defence factors. This would account for the failure of *Chlamydia* detection in tissue culture in spite of its presence in the specimen.

As opposed to gonoccocal infection, which has greatly decreased, *C. trachomatis* cervical infection is common in the young. Our report suggests that prevalence may be age-dependent with women under 25 having the higher risk ($p = 0.007$). The very

alarming figure of 33.3% prevalence rate amongst the subfertile women less than 25 years old compared with the rate of 4.5% in the women more than 25 years old cannot be ignored although a larger sample size would be necessary before a more definite conclusion can be drawn. The authors admit that the numbers are indeed small and statistical conclusions should be duly interpreted. The magnitude of this "hidden" epidemic has been highlighted by many authors too[2,6]. More than 30 different studies covering 200 – 12,000 subjects screened in family planning centres, college women, students and military recruits in different parts of the USA, in Scandinavian countries and France, indicate a prevalence of 5% – 20% (mean 10%), in apparently healthy young females less than 25 years old with the highest (15% – 20%) in women less than 20 years old[10]. Several cost-benefit analysis show that the total cost of the general screening in young populations, could save twice the cost of treatment for pelvic inflammatory disease caused by *C trachomatis* and six times the total cost of *C trachomatis* epidemics if late sequelae are taken into account (tubal infertility treatment and ectopic pregnancy). This is not surprising as an effective protocol for treatment involves administrating oral doxycline 100 mg, twice daily for seven days.

The simplicity and inexpensive nature of treatment may prompt some clinicians to doubt the necessity of screening since such empirical prophylaxis using antibiotics is readily available and relatively cheap. However, we must not forget the true purpose of screening this group of patients because the accurate diagnosis of asymptomatic infection can facilitate identification, treatment, and education of their sexual partners. With this view, we can recommend that the screening of lower genital tract carriage of *Chlamydia trachomatis* with the treatment of positive cases is a preferable alternative.

In summary, we find that the reservoir of infected asymptomatic individuals who make up the bulk of prevailing infections is significant in our group of subfertile patients. More importantly, they are responsible for maintaining transmission of the infection within the community.

Coupled by the fact that majority are in the less than 25-year-old age range, the far reaching consequences of continued sexual transmission of this innoculous organism cannot be neglected. We feel that the time is right, with this proper diagnostic tool, to embark on a larger-scale screening programme. In the most recent recommendation, arising from the Study Group on the Prevention of Pelvic Infection, the Royal College Of Obstetricians and Gynaecologists emphasised the need to address the issue of optimal frequency of such screening in both high and low risk groups, and the importance of developing strategies for gaining access for screening groups of patients with a *chlamydia* prevalence of 3% to 6% (prevalence rates for which it has been suggested that *chlamydia* screening will be cost effective). Furthermore, there should be increased health awareness and education to ensure the efficacy of any screening programme. There is also an immediate need for long term research projects to assess the efficacy of screening for *Chlamydia trachomatis* infection, including assessment of rates of hospital admissions for PID, ectopic pregnancy and tubal infertility.

CONCLUSION

Surveillance of genital *chlamydia* infection is often guided by two important aspects: (1) their long term impact on public health, and (2) their incidence in various population subgroups. We see an alarming proportion of the subfertile women in our society harbouring the *chlamydia* infection especially in the below 25-year-old age group. Although, this may represent the end of the spectrum of clinical consequences of this diseases, in this group of women with infertility, it is still important to identify this group for treatment, counselling and contact tracing. It is only through such measures that hospitalisations due to pelvic inflammatory diseases, and ectopic pregnancies can be reduced. Having diagnosed the infection will also help the reproductive gynaecologist plan the most suitable approach to helping the couple achieve a pregnancy without any of the infective consequences of genital *Chlamydia trachomatis*.

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